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Mini-Review

Predictive Gestational Diabetes Biomarkers With Sustained Alterations Throughout Pregnancy

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Abstract

Gestational diabetes mellitus (GDM) results in an increased risk of pre- and postpartum health complications for both mother and child. Metabolomics analysis can potentially identify predictive biomarkers and provide insight into metabolic alterations associated with GDM pathogenesis and progression, but few metabolomics studies investigate alterations observed across the first and third trimester. We hypothesize that metabolites altered in first-trimester GDM that remain altered in late pregnancy may best inform interventions. Metabolomic studies comparing plasma and serum metabolite alterations in GDM vs non-GDM pregnancies were retrieved by searching PubMed, Medline, and CINAHL Plus databases. The present scoping review summarizes the metabolites found to be consistently altered throughout the course of GDM and proposes mechanisms that explain how these metabolic perturbations relate to GDM development and progression. Metabolites involved in fatty acid metabolism, reductive carboxylation, branched-chain amino acid metabolism, cell membrane lipid metabolism, purine degradation, and the gut microbiome were found to be altered throughout GDM pregnancies, with many of these pathways showing mechanistic links to insulin resistance, inflammation, and impaired cell signaling. Future studies are required to investigate if normalization of these perturbed pathways can be the targets of interventions.

Key Words: gestational diabetes mellitus, metabolomics, fatty acid metabolism, reductive carboxylation, branched-chain amino acids, purine degradation

Abbreviations: BCAA, branched-chain amino acid; EPA, eicosapentaenoic acid; FAO, fatty acid oxidation; FFA, free fatty acid; GDM, gestational diabetes mellitus; LPC, lysophosphatidylcholine; PC, phosphatidylcholines; PLA2, phospholipase A2; SM, sphingomyelin; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TMAO, trimethylamine n-oxide; XO, xanthine oxidase.

Gestational diabetes mellitus (GDM) is a metabolic disorder defined as glucose intolerance first identified during pregnancy and is characterized by hyperglycemia and insulin resistance. The prevalence of GDM varies worldwide, currently estimated at 7% to 10% [1], although it is difficult to pinpoint due to unstandardized screening and diagnostic criteria. GDM increases the risk of negative health outcomes for mother and child, occurring both during and after pregnancy. There is significant evidence that pregnancies affected by GDM are at an increased risk of cesarean section, preeclampsia, macrosomia, and neonatal hypoglycemia [2]. Furthermore, GDM has been found to increase the long-term risk of metabolic complications, such as type 2 diabetes mellitus (T2DM) and obesity for the mother and child [3].

In non-GDM pregnancy, glucose metabolism is also altered to meet the changes in energy demands. There is a progressive decline in insulin sensitivity of about 40% to 50%. Consequently, to maintain euglycemia, β-cells compensate by increasing insulin secretion by 200% to 250% [4]. Hepatic glucose production along with fasting insulin increase into late pregnancy, which could explain the decrease in insulin sensitivity in normoglycemic patients [5]. While the pathophysiology of GDM has not been fully elucidated, various factors have been identified that exacerbate glucose intolerance and insulin resistance during pregnancy. Obesity is an established risk factor of GDM, and dysregulated adipocytes lead to an increase in the production of proinflammatory cytokines, which results in chronic low-grade inflammation [6]. Several studies have observed elevated levels of proinflammatory cytokines, interleukin-6, and tumor necrosis factor α, in the plasma and serum of patients with GDM [7–10]. Another potential contributory factor is altered β-cell dysfunction, leading to insufficient insulin production to meet metabolic needs during pregnancy. β-Cells release insulin to maintain euglycemia through a cascade of events that result in translocation of glucose transporter type 4 to the cell surface to allow glucose uptake [2, 11]. GDM may be the result of a chronic β-cell defect that had been undetected until pregnancy [12], with a small percentage associated with the presence of β-cell autoantibodies or monogenic diabetes [2, 12].

Although numerous metabolomics studies have identified biomarkers potentially predictive of GDM, findings have

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not yet been sufficient to translate into clinical use [13]. Few studies have tracked biomarker changes across pregnancy in order to prospectively determine whether and how they changed [14–17], making it difficult to assess metabolic alterations associated with GDM pathogenesis and progression. First-trimester metabolite alterations differentiating mothers who develop GDM from mothers who do not develop GDM, and that remain altered throughout pregnancy, may be particularly important as potential future targets for intervention [18, 19]. With this concept in mind, this scoping review focuses on biomarkers associated with GDM prediction that remain altered throughout the course of pregnancy. We postulate that these biomarkers may best inform future interventions.

Literature Search Methods

For this scoping review, metabolomic studies comparing metabolite alterations in GDM vs non-GDM pregnancies were retrieved by searching the PubMed, Medline, and CINAHL Plus databases. Search terms included gestational diabetes AND metabolomics. Only metabolomics studies utilizing fasting and nonfasting plasma or serum samples were included. All other sample types were excluded to allow for more accurate comparison of metabolite alterations between studies, as sample types such as urine, feces, and tissue samples all feature very different metabolic profiles and therefore would be unlikely to have overlapping results [20]. Plasma or serum samples were selected for inclusion due to being the most common sample type used in GDM metabolomics studies (Fig. 1), while also having only minor differences in metabolic profiles [20]. No studies were excluded on the basis of gravidity, parity, maternal age, or weight history.

Studies across all trimesters were included and are defined in this review as either prediagnosis studies, at-diagnosis studies, or postdiagnosis studies. Prediagnosis GDM studies include studies that collected blood samples prior to GDM diagnosis, with sample collection times ranging between 2 and 21 weeks of gestation. At-diagnosis GDM studies include studies that collected blood samples taken on the same day that GDM diagnosis occurred, with sample collection times ranging between 22 and 28 weeks of gestation. Postdiagnosis GDM studies includes studies that collected blood samples after GDM diagnosis, with sample collection times ranging between 28 and 38 weeks of gestation and typically occurring between 3 and 7 weeks after diagnosis. Metabolites altered between GDM and non-GDM groups with $P < 0.05$ were considered statistically significant, and thus considered for inclusion in this review. Metabolite concentrations that changed in the same direction in at least 2 prediagnosis and at least 1 at-diagnosis or postdiagnosis studies, or at least 1 prediagnosis and at least 2 at-diagnosis or postdiagnosis studies were included in this review.

Metabolic Pathways Dysregulated Across GDM Pregnancy

Metabolic pathways observed to be dysregulated across GDM pregnancy were fatty acid metabolism, reductive carboxylation, branched-chain amino acid (BCAA) metabolism, and cell membrane lipid metabolism (Fig. 2).

Fatty Acid Metabolism

Dysregulated fatty acid metabolism was seen across pregnancy, with alterations in ketone bodies, fatty acids, and alpha-hydroxybutyrate indicating upregulation of fatty acid oxidation (FAO) and ketogenesis.

The ketone body acetoacetate was elevated in 2 prediagnosis [21, 22], 2 at-diagnosis studies [21, 23], and 1 postdiagnosis study [24] indicating an increase in ketogenesis. In support of altered ketone body metabolism is the observed increase of β-hydroxybutyrate in 1 prediagnosis study [25], 2 at-diagnosis studies [21, 26], and 1 postdiagnosis study [24]. Oleic acid, a monounsaturated fatty acid, was found to be elevated in 1 prediagnosis [27], 1 at-diagnosis [28], and 2 postdiagnosis studies [14, 28], and the saturated fatty acid palmitic acid was elevated in 1 prediagnosis [14], 2 at-diagnosis [23, 28], and 2 postdiagnosis studies [14, 28]. Myristic acid, another saturated fatty acid, was elevated in 2 prediagnosis [14, 27] and 1 postdiagnosis study [14] (Table 1). As seen in T2DM, insulin resistance may result in the utilization of ketone bodies for energy production due to low glucose availability [29]. Because ketone bodies are products of FAO, the increase in plasma fatty acids may be reflective of this increase in ketogenesis (Fig. 3). This is supported by the observed increase of glycerol in 3 at-diagnosis studies [23, 26, 28], and 1 postdiagnosis study [28], as ketogenesis begins with lipolysis resulting in a breakdown of triglycerides into free fatty acids (FFAs) and glycerol to provide FFAs as ketone body building blocks [30]. Elevation of ketone bodies is a marker of poorly controlled diabetes [30], so their continued elevation into late pregnancy indicates poorly managed GDM. However, elevation of these ketone bodies was noted in at-diagnosis studies but not in postdiagnosis studies, suggesting that postdiagnosis GDM treatment may normalize ketone body levels.

In spite of corrected ketone body concentrations, the persistent elevation of fatty acids even after diagnosis points to a continued dysregulation of fatty acid metabolism that is not fully ameliorated by GDM management. Once elevated, FFAs have been found to inhibit insulin's antilipoptotic mechanism, resulting in increased lipolysis and continued elevation of FFAs [31]. The increase in plasma fatty acids in early pregnancy GDM for the purpose of ketogenesis may therefore work to maintain elevated fatty acid levels in late pregnancy, even after ketogenesis is no longer in use due to improved control of blood glucose levels. Elevated FFAs have been found to contribute to insulin resistance, potentially through the generation of proinflammatory cytokines and oxidative and endoplasmic reticulum stress [31]. As such, the continued elevation of fatty acids into late pregnancy may contribute to the severity of GDM, though further research is required.

In further support of dysregulated fatty acid metabolism is the observed alterations in alpha-hydroxybutyrate, a metabolite closely connected to FAO. Alpha-hydroxybutyrate was elevated in 1 prediagnosis [25], 3 at-diagnosis [23, 26, 28], and 1 postdiagnosis studies [15] (Table 1). Alpha-hydroxybutyrate has been identified as a marker of the elevated ratio of cytosolic NADH/NAD^+, as this metabolite is formed from alpha-ketobutyrate in a reaction that can be catalyzed by lactate dehydrogenase when the cytosolic NADH/NAD^+ ratio is increased [49, 50] (Fig. 3). In support of this altered pathway is the observed increase in lactate in 1 prediagnosis [27], 1

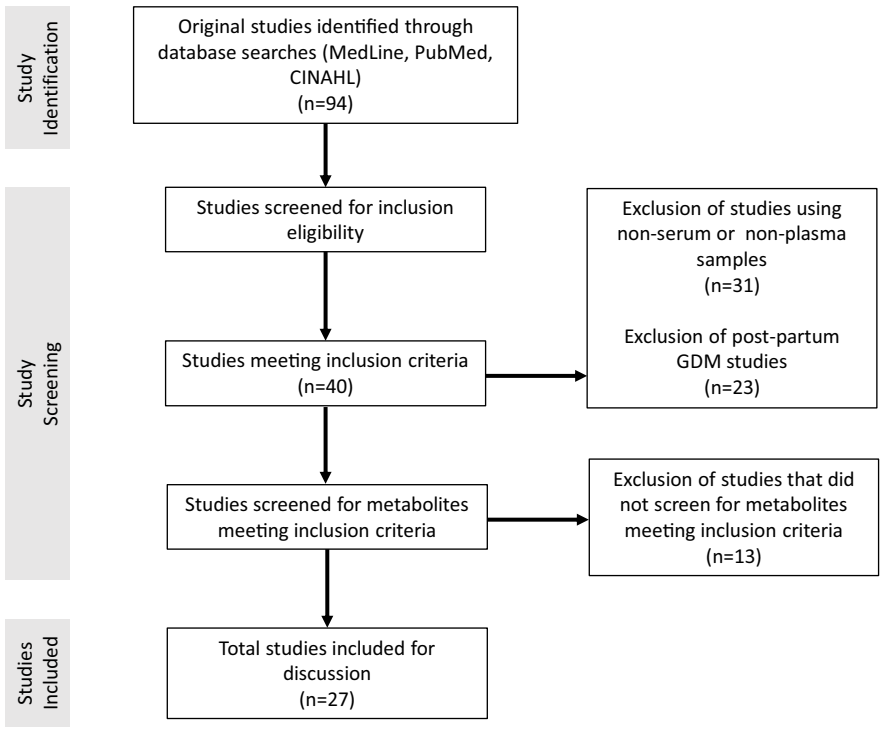

Figure 1. Study and metabolite search flowchart.

at-diagnosis [23], and 1 postdiagnosis study [32], as elevated lactate/pyruvate ratio has been associated with high alpha-hydroxybutyrate and high NADH/NAD$^+$ ratio [50]. The NADH/NAD$^+$ ratio is proposed to be increased during elevated FAO triggered by increased insulin resistance [49]. Alpha-hydroxybutyrate is associated with impaired glucose tolerance and insulin resistance in people with T2DM [51] and has been found to predict dysglycemia in nondiabetic populations [49, 52]. It has also been proposed as a potential early biomarker of GDM [25]. The elevation of alpha-hydroxybutyrate throughout GDM-affected pregnancy indicates persistent insulin resistance and may be related to continued reliance on FAO throughout pregnancy, which increases the NADH/NAD$^+$ ratio. This is in line with the previously discussed increase in FFAs in late pregnancy. It has been proposed that increased FAO oxidation results in decreased glucose oxidation in a process that contributes to insulin resistance [53], indicating that GDM management is not effectively facilitating the switch from FAO to glucose metabolism. There may be a few reasons for this, one being that there is currently little consensus regarding dietary macronutrient distribution during GDM management, with some recommendations encouraging decreased carbohydrate intake and up to 40% of energy intake from fats [54]. It is possible that elevated fat intake during GDM further contributes to increased FAO and elevated alpha-hydroxybutyrate. Additionally, because FFAs are elevated in early pregnancy, prior to diagnosis and intervention, and this increase then may contribute to their sustained elevation in late pregnancy [31], this indicates that the decrease in FAO and alpha-hydroxybutyrate may be achieved by intervening during early pregnancy, but not during late pregnancy. Further research is required to investigate whether earlier intervention would result in decrease alpha-hydroxybutyrate and improved outcomes.

Reductive Carboxylation

Pyruvate, the end-product of glycolysis, was elevated in 5 prediagnosis [21, 22, 33–35] and 2 at diagnosis studies [21, 23]. One postdiagnosis study observed a decrease in pyruvate [35]. Similarly, citrate was found to be higher in in 3 prediagnosis studies [15, 21, 22], 1 at-diagnosis study [21], and 1 postdiagnosis study [15] (Table 1). Pyruvate and citrate have been implicated in glucose-stimulated insulin secretion, with pyruvate working to maintain high citrate levels via the reductive carboxylation pathway for the purpose of NADPH production, which supports insulin secretion [55, 56] (Fig. 3). This is further supported by the decrease in glutamine in 3 prediagnosis studies [21, 22, 38], 3 at-diagnosis studies [21, 38, 39], and 1 postdiagnosis GDM study [35], as well as the an increase in glutamate in 1 prediagnosis [36] and 2 at-diagnosis GDM studies [23, 37]. Glutamine, via conversion to glutamate, can feed into the tricarboxylic cycle for the purpose of citrate production [56]. Increased glucose-stimulated insulin secretion is a common metabolic adaptation observed in conditions of insulin resistance [57]. Additionally, reductive carboxylation is utilized by impaired mitochondria in tumor cells to supply citrate and acetyl-CoA for the purpose of fatty acid synthesis [58]. Mitochondrial impairment is also a feature of diabetes, making reductive carboxylation a possible supporter of the previously discussed elevation in FAO via the synthesis of fatty acids (Fig. 3). The increase in pyruvate and citrate in GDM may be a way of compensating for the GDM-associated increase in insulin resistance. The dysregulation of this pathway throughout pregnancy may indicate that insulin resistance develops early on in GDM pregnancies, and that current GDM management does not adequately treat this insulin resistance. Prepregnancy intervention, particularly for those at risk of GDM, may normalize this pathway before it becomes dysregulated to the point of GDM development.

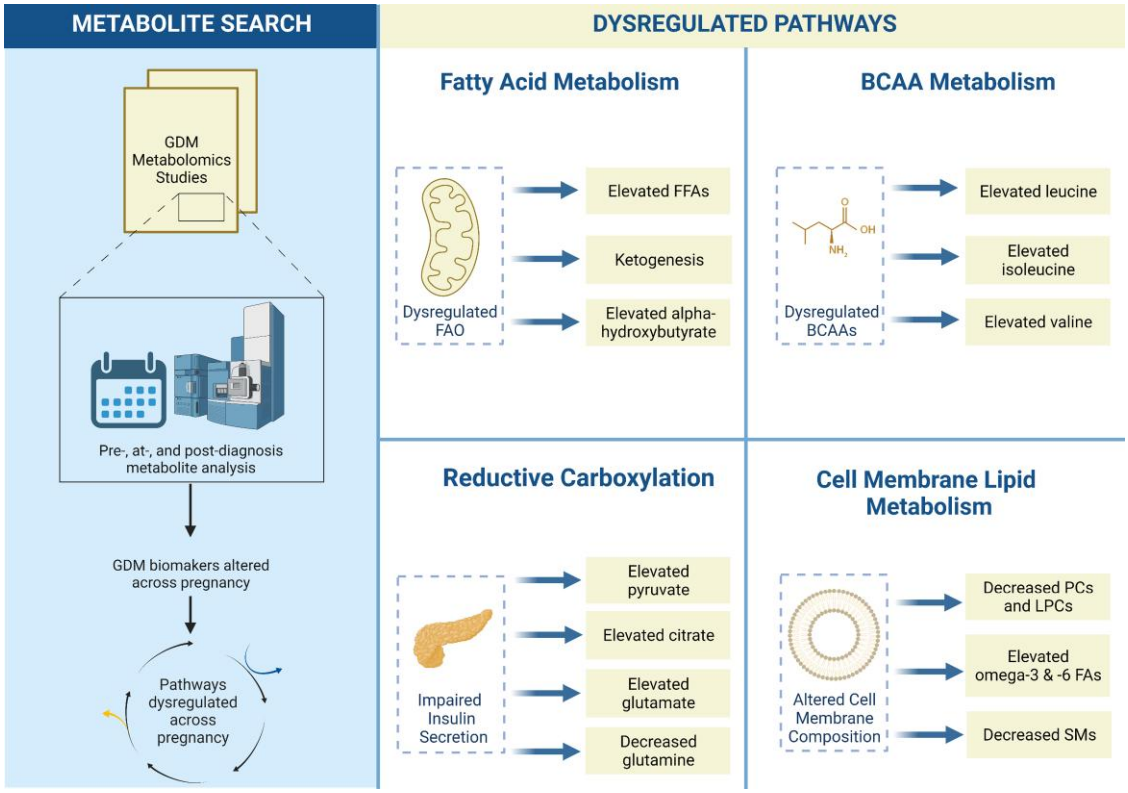

Figure 2. A summary of metabolites and pathways found to be altered across pregnancy in GDM metabolomic studies. Pathways observed to be dysregulated across pregnancy in GDM metabolomics studies include fatty acid metabolism, BCAA metabolism, reductive carboxylation, and cell membrane lipid metabolism. Figure created in BioRender.com. FA, fatty acid; FAO, fatty acid oxidation; FFA, free fatty acids; BCAA, branched-chain amino acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelins.

Exercise, dietary interventions, and weight loss have all been shown to improve insulin sensitivity and glycemic control among people with insulin resistance and T2DM [59–62], suggesting that these methods may be appropriate prepregnancy interventions to reduce GDM occurrence by maintaining appropriate glucose-stimulated insulin secretion levels.

Branched-chain Amino Acid Metabolism

BCAAs were upregulated across pregnancy in GDM studies, with leucine increasing in 5 prediagnosis [21, 22, 33, 34, 41] and 4 postdiagnosis studies [21, 23, 42, 43], isoleucine increase in 4 prediagnosis [21, 22, 33, 34] and 2 at-diagnosis studies [21, 23], and valine increasing in 5 prediagnosis studies [21, 22, 27, 33, 35], 2 at-diagnosis studies [21, 23], and 1 postdiagnosis study [15]. Both isoleucine and valine were found to decrease in 1 prediagnosis study [17], while valine also decreased in 1 at-diagnosis [44] and 1 postdiagnosis study [35] (Table 1). Elevated BCAAs have previously been identified as markers of insulin resistance, with a study by Wang et al. finding elevated BCAAs to indicate a 4-fold increased risk of developing T2DM, while also being elevated up to 12 years prior to onset of T2DM [63]. The mechanistic relationship between elevated BCAA and insulin resistance remains unclear, but it has been proposed that persistent elevation of BCAAs activates the mTORC1 signaling pathway, thereby promoting insulin resistance through the increased phosphorylation of insulin receptor substrate 1 and 2 [64]. Conversely, it may be that some people present with a phenotype of dysregulated BCAA metabolism, resulting in a build-up of toxic BCAA metabolites that induces β-cell apoptosis and insulin resistance [65]. In a clinical trial of insulin vs metformin treatment for women with GDM, a significant increase in isoleucine, and a trend increase in leucine, was observed in metformin compared with insulin treatment [66]. As such, metformin treatment for GDM may contribute to BCAAs remaining elevated even in postdiagnosis GDM. From a dietary intervention standpoint, reducing intake of BCAAs lowers BCAA levels in the blood and improves insulin resistance [67, 68], and weight loss through caloric restriction has similar effects [69]. However, neither reduced BCAA intake nor weight loss through caloric restriction is a GDM management recommendation in late pregnancy [70], potentially further explaining the sustained elevation of BCAAs in postdiagnosis GDM. A past study of women with a recent history of GDM found that elevated BCAAs were highly predictive of the transition from GDM to T2DM [71], indicating that persistent elevation of BCAAs during and after GDM pregnancy may serve as a marker of dysregulated energy metabolism and diabetes risk.

Cell Membrane Lipids

The cell membrane lipids, phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), and sphingomyelins (SMs), were decreased across GDM-affected pregnancies. Although the reason has not been deciphered, this may be related to dysregulated use of lipid mediators such as oxylipins and ceramides, as well as of lipid rafts, all of which are associated with inflammation, cell signaling, and insulin resistance.

Table 1. Metabolites altered across pregnancy in GDM metabolomics studies

Metabolite	Metabolic pathway	Metabolite class	Changes in prediagnosis GDM	Changes in at-diagnosis GDM	Changes in postdiagnosis GDM
Acetoacetate	Fatty acid metabolism	Ketone body	↑ [21, 22]	↑ [21, 26]	↑ [24]
Beta-hydroxybutyrate	Fatty acid metabolism	Ketone body	↑ [25]	↑ [21, 23]	↑ [24]
Oleic acid	Fatty acid metabolism	Monounsaturated fatty acid	↑ [27]	↑ [28]	↑ [14, 28]
Palmitic acid	Fatty acid metabolism	Saturated fatty acid	↑ [14]	↑ [23, 28]	↑ [14, 28]
Myristic acid	Fatty acid metabolism	Saturated fatty acid	↑ [14, 27]		↑ [14]
Alpha-hydroxybutyrate	Fatty acid metabolism	Hydroxybutyric acid	↑ [25]	↑ [23, 26, 28]	↑ [15]
Lactate	Fatty acid metabolism	Organic acid	↑ [27]	↑ [23]	↑ [32]
Pyruvate	Reductive carboxylation	Alpha-keto acid	↑ [21, 22, 33–35]	↑ [21, 23] ↓ [26]	
Citrate	Reductive carboxylation	Tricarboxylic acid	↑ [15, 21, 22]	↑ [21]	↑ [15]
Glutamate	Reductive carboxylation	Amino Acid	↑ [36]	↑ [23, 37]	
Glutamine	Reductive carboxylation	Amino acid	↓ [21, 22, 38]	↓ [21, 38, 39] ↑ [23]	↓ [35] ↑ [40]
Leucine	BCAA metabolism	BCAA	↑ [21, 22, 33, 34, 41]		↑ [21, 23, 42, 43]
Isoleucine	BCAA metabolism	BCAA	↑ [21, 22, 33, 34] ↓ [17]	↑ [21, 23]	
Valine	BCAA metabolism	BCAA	↑ [21, 22, 27, 33, 35] ↓ [17]	↑ [21, 23] ↓ [44]	↑ [15] ↓ [35]
PCs	Cell membrane lipid metabolism	Phospholipid	↓ [45]	↓ [26, 43]	
LPCs	Cell membrane lipid metabolism	Lysophospholipid	↓ [17, 45]	↓ [26]	↓ [32]
EPA	Cell membrane lipid metabolism	Omega-3 fatty acid	↑ [14]	↑ [46]	↑ [14]
Linoleic Acid	Cell membrane lipid metabolism	Omega-6 fatty acid	↑ [27]	↑ [26, 28]	↑ [14]
SMs	Cell membrane lipid metabolism	Sphingolipid	↓ [25, 45] ↑ [25]	↓ [26]	↓ [32]
Betaine	Gut microbiome	Amino acid derivative	↓ [47, 48]		↓ [35]
TMAO	Gut microbiome	Amine oxide	↓ [47, 48]		↓ [35]

Prediagnosis GDM includes studies that collected blood samples prior to GDM diagnosis, with sample collection times ranging between 2 and 21 weeks of gestation. At-diagnosis GDM includes studies that collected blood samples taken on the same day that GDM diagnosis occurred, with sample collection times ranging between 22 and 28 weeks of gestation. Postdiagnosis GDM includes studies that collected blood samples after GDM diagnosis, with sample collection times ranging between 28 and 38 weeks of gestation and typically occurring between 3 and 7 weeks after diagnosis.
Abbreviations: BCAA, branched-chain amino acid; EPA, eicosapentaenoic acid; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin; TMAO, trimethylamine n-oxide.

LPCs decreased in 2 prediagnosis studies [17, 45], 1 at-diagnosis study [26], and 1 postdiagnosis study [32], while PCs decreased in 1 prediagnosis [45] and 2 at-diagnosis studies [26, 43]. In support of these alterations was the observed increase in the omega-3 fatty acid eicosapentaenoic acid (EPA) in 1 prediagnosis [14], 1 at-diagnosis [46], and 1 postdiagnosis study [14]. The omega-6 fatty acid linoleic acid was also found to increase in 1 prediagnosis study [27], 2 at-diagnosis studies [26, 28], and 1 postdiagnosis study [14] (Table 1). Fatty acids can be freed from plasma membranes by the enzyme family phospholipase A2 (PLA2), resulting in a release of fatty acids and LPCs from phospholipids, such as PCs [72] (Fig. 4). Increased activity of PLA2 has been associated with elevated blood glucose and insulin resistance, though current studies are limited to animal models and correlations, making it difficult to determine any mechanist relationships that may exist between PLA2 and insulin resistance [73–75]. However, the observed decrease in PCs and increase in omega-3 and omega-6 fatty acids in GDM may be a result of increased PLA2 activity. Once freed from the plasma membrane by PLA2, LPCs can be hydrolyzed into lysophosphatidic acid by the enzyme autotaxin (Fig. 4). Elevated autotaxin and lysophosphatidic acid has been associated with insulin resistance [76], indicating that LPCs may be depleted in GDM through the action of autotaxin. The decrease in plasma membrane phospholipids in GDM can alter cell membrane fluidity [45], which may impair glucose uptake and insulin binding, therefore contributing to insulin resistance [77].

In addition to potentially altering membrane fluidity, increased PLA2 activity can also result in elevated oxylipins (Fig. 4), with oxylipins derived from omega-6 fatty acids being inflammatory and those derived from omega-3 fatty acids being predominantly anti-inflammatory [78]. While EPA

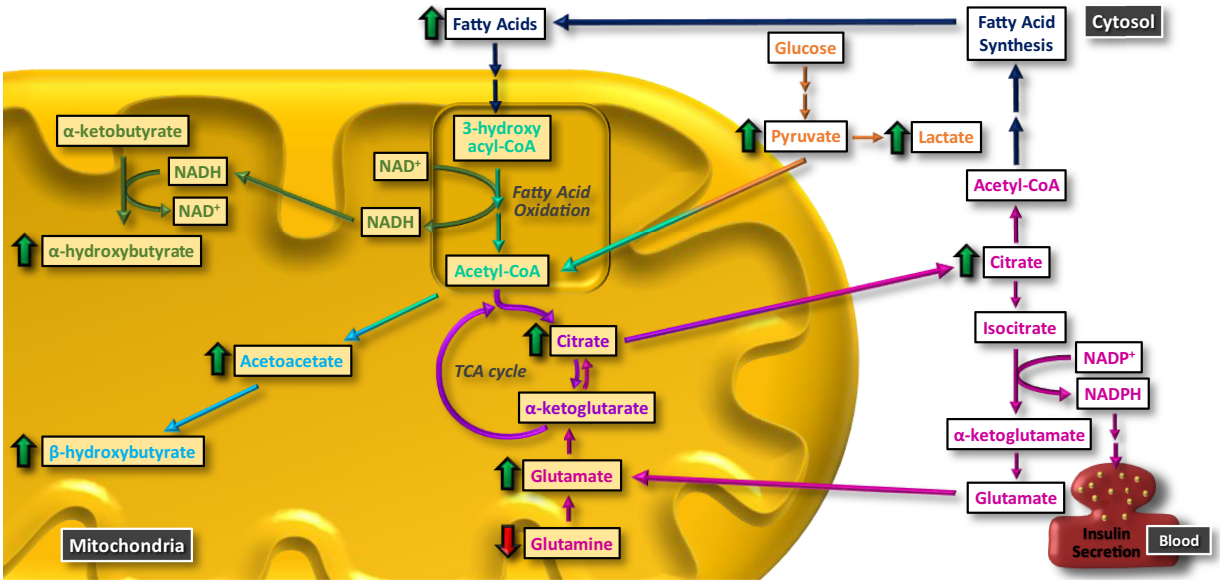

Figure 3. Fatty acid metabolism and reductive carboxylation metabolites observed to be altered throughout GDM-affected pregnancies. Upward arrows indicate an increase in the metabolite in GDM compared with non-GDM pregnancies while downward arrows indicate a decrease.

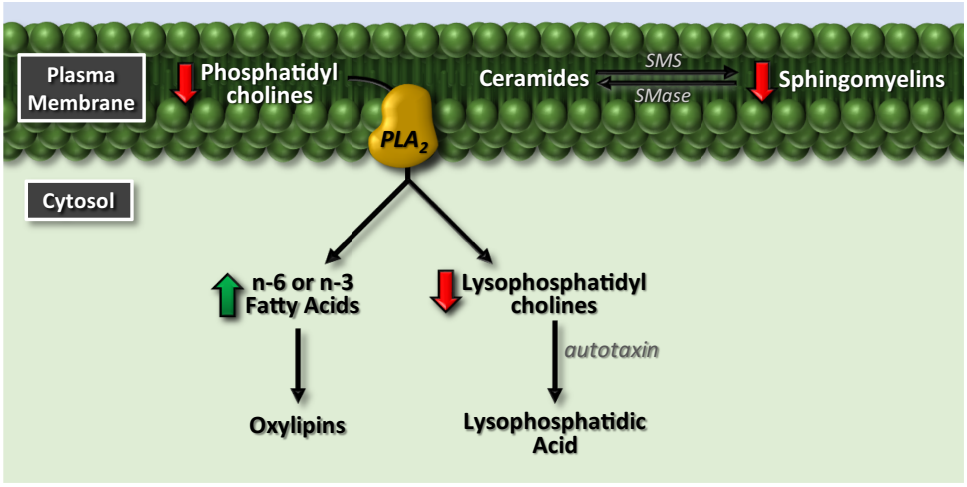

Figure 4. Cell membrane lipid metabolites observed to be altered throughout GDM-affected pregnancies. Upward arrows indicate an increase in the metabolite in GDM compared to non-GDM pregnancies while downward arrows indicate a decrease.

mainly produces anti-inflammatory oxylipins, such as resolvins, it can also produce oxylipins with some inflammatory properties [78, 79]. As such, the elevation of EPA in GDM may not be enough to combat the inflammation arising from linolenic acid–derived oxylipins. Due to the association between inflammation and insulin resistance, the dysregulation of omega-6 fatty acids and omega-3 fatty acids and their resulting oxylipins may contribute to GDM. Unfortunately, to our knowledge, no studies have investigated potential alterations in oxylipins due to GDM. Therefore, future GDM studies conducting oxylipin analysis are needed to confirm this occurrence.

Exercise, a conventional GDM management technique, has not been found to result in a reduction in PLA2 activity [80], which may explain why this pathway continues to be dysregulated even after diagnosis. The Western diet has been found to result in increased lipoprotein-PLA2 activity compared with healthier, more Mediterranean diet [81], but dietary intervention for people with GDM is not standardized [54], so it is possible that certain dietary recommendations do not adequately alter PLA2 activity. Additionally, it may be that the limited amount of time between GDM diagnosis and delivery is not sufficient to allow for any metabolic improvements from dietary changes. However, the link between PLA2 activity, oxylipins, and GDM remains unclear, as there is a lack of knowledge concerning changes in oxylipins associated with GDM. As previously mentioned, additional GDM studies are required to verify potential alterations in these lipid mediators.

SMs were observed to decrease in 1 prediagnosis [45], 1 at-diagnosis [26], and 1 postdiagnosis study [32]. One prediagnosis biomarker study found SMs to both increase and decrease,

though the SMs with the highest diagnostic accuracy were observed to decrease [25]. The downregulation of sphingolipid metabolism among those with GDM has been found to be strongly associated with an increased risk of developing T2DM [71, 82]. A knockout model of sphingomyelin synthase, the enzyme that converts ceramides to SMs, has been observed to result in a build-up of ceramides, which then induce mitochondrial dysfunction and impair insulin secretion [83]. Build-up of ceramides has also been found to impair Akt/PKB phosphorylation, which is an important step in the insulin signaling cascade. As such, a reduction in SMs may indicate an increase in ceramides that contributes to insulin resistance [84]. Furthermore, SMs are an important component of plasma membrane lipid rafts, which have been shown to be involved in cell signaling. Alterations in lipid rafts may play a role in insulin resistance pathogenesis [85], indicating that the decrease in SMs, potentially due to ceramide build-up, may further contribute to GDM development through the dysregulation of lipid raft functions. However, ceramides were not detected in the reviewed GDM metabolomics studies, highlighting the need for further investigation of GDM, SMs, and ceramides. Exercise, as well as exercise-induced weight loss, has been observed to reduce muscular ceramide levels while also correlating with improved insulin sensitivity [86, 87]. While moderate-intensity exercise is recommended for those with GDM [54, 70], the timing of GDM diagnosis and subsequent management, which occurs in late pregnancy, may result in the intervention period being too short to see any improvement in SMs. Earlier diagnosis and earlier intervention methods could potentially ameliorate alterations in SMs, though further investigation is required to determine if this would impact GDM occurrence or severity.

Other Metabolic Pathways Altered

Two other metabolic pathways, purine degradation and microbiome-associated metabolites, were observed to be altered across pregnancy. While information regarding these alterations are less robust than the previously discussed pathways, they are included in this review due to meeting our inclusion criteria while displaying some connections to insulin resistance.

Several purine degradation metabolites were upregulated in GDM up to the second trimester of pregnancy. Hypoxanthine was elevated in 2 prediagnosis studies [17, 25] and in 1 unpublished postdiagnosis study (Heath, Phelan, La Frano; personal communication), xanthine was elevated in 2 prediagnosis studies [25, 88] and in 1 at-diagnosis study [89], and uric acid increased between the first and second trimesters in a prediagnosis study [17]. Increased levels of xanthine in serum during early pregnancy may be predictive of GDM. Tian et al. identified significantly increased levels of xanthine during the first trimester of subjects who were later diagnosed with GDM [88] and in another study xanthine plasma levels were significantly higher in women with GDM during their second trimester and postpartum when compared with control subjects [89]. Xanthine oxidase (XO) converts hypoxanthine to xanthine and xanthine to uric acid, and produces reactive oxygen species that have been associated with increased inflammation and insulin dysregulation [90]. The observed elevation of purine degradation products in subjects with GDM suggests impaired XO activity [88]. This is further supported by 2 studies that found increased levels of XO in the serum, placenta, and cord plasma of subjects with GDM, and there was a positive correlation between XO levels and HbA1C [91, 92]. Upregulation of XO activity is supported by various studies that noted elevated levels of uric acid to be associated with insulin resistance or increased risk of GDM or T2DM [93–95]. In a study of nucleotide and nucleoside levels in erythrocytes, subjects with type 1 diabetes mellitus (T1DM) and T2DM had significantly higher levels of purine nucleotides, nucleosides, and purine degradation products. This suggests a relationship between increase nucleotide synthesis and degradation and hyperglycemia. Several studies have found associations between the elevation of the nucleoside adenosine and GDM [96, 97]. It is proposed that elevated levels of adenosine may be due to decreased expression of human equilibrative nucleoside transporter 1 (hENT1). Increased levels of adenosine may upregulate nitric oxide synthesis and alter blood flow via the umbilical vein [97].

The current aim of GDM treatment is to maintain euglycemia. Common treatments consist of lifestyle modifications or pharmacological intervention, with a reported 70% to 85% people successful controlling their GDM with lifestyle changes alone [98]. However, studies examining the changes GDM interventions have on purine degradation products are lacking. In a study of patients with obesity who underwent bariatric surgery, serum uric acid levels significantly decreased, and a reduction of XO activity was observed. However, researchers did not identify an association between decreased XO activity and the reduction in uric acid levels. Additionally, no correlation was found between changes in uric acid and improved insulin resistance after bariatric surgery. Reduction in uric acid did correlate with lower triglyceride levels [99]. In trained individuals, with a lifelong history of performing high-intensity exercise, purine metabolites, hypoxanthine, xanthine, and uric acid, were lower than in untrained individuals. This suggests that high-intensity exercise may reduce purine degradation in trained skeletal muscle [100]. While high-intensity exercise is not be advisable during pregnancy, mild-intensity exercise is often prescribed. Regarding the latter, it is promising to note that a study of mild-intensity exercise in elderly subjects did find a reduction in serum hypoxanthine levels [101].

Betaine increased in 2 prediagnosis studies [47, 48] and 1 postdiagnosis study [35], while trimethylamine n-oxide (TMAO) decreased in 2 prediagnosis studies [47, 48] and 1 postdiagnosis study [35]. The decrease in TMAO appears counterintuitive, as elevated TMAO has previously been associated with both GDM and insulin resistance [102, 103]. While the decrease in TMAO is unexpected and requires further research, both TMAO and betaine are associated with the gut microbiome and thereby may be indicative of its alteration in general. The gut microbiome has been found to be disturbed in those with GDM [104–106], and gut microbiome dysbiosis has also been linked to the development and progression of diabetes [107]. While probiotics have not been effective in treating GDM [108], a rat model of GDM found that exercise prior to and during pregnancy prevented alterations of the gut microbiome as well as the development of metabolic dysregulation [109]. However, exercise during only pregnancy was not effective, which may explain why TMAO and betaine were altered across pregnancy, even after the potential increase in physical activity that is recommended upon GDM diagnosis. The implementation of both pre- and postpregnancy intervention may maintain a healthy gut microbiome

and therefore keep gut microbiome metabolites at normal levels, though further research in human studies is necessary.

Another potential reason for the observed changes in choline and betaine may relate to the previously discussed decrease in PCs across pregnancy [26, 43, 45]. PCs may be broken down into choline and phosphatidic acid via the action of phospholipase D [110], and choline could then contribute to elevated betaine [111]. Elevated phosphatidic acid may play a role in mTORC1 signaling, thereby contributing to insulin resistance [110, 112], while betaine is important for methylation via the action of betaine-homocysteine S-methyltransferase [111]. Dysregulated phospholipase D signaling, and hypermethylation that alters genomic regions related to insulin resistance, has been observed in GDM [113]. Furthermore, elevated betaine-homocysteine S-methyltransferase mRNA has been observed in diabetic rats [111], suggesting the involvement of betaine in insulin resistance development via alterations in methylation, though further research is necessary to fully understand this connection.

Strengths, Limitations, and Future Directions

This review has several strengths, particularly in the inclusion of metabolites altered across pregnancy. Few longitudinal GDM metabolomics studies exist, which limits the observation of metabolites associated with GDM development from first to third trimester, and metabolites that are potentially resistant to current GDM management methods. As such, our inclusion of metabolites across all pregnancy stages allows for the exploration of metabolic perturbations related to sustained metabolite alterations, therefore providing insight into underlying mechanisms of GDM development and severity. However, this review is limited by several factors relating to current GDM metabolomic study designs.

Metabolites can be affected by extrinsic factors such as diet and exercise, but these variables were rarely controlled for in the reviewed studies. The often small sample sizes and lack of longitudinal studies further limits our ability to gain a full perspective of metabolic alterations occurring across pregnancy. Another large challenge is posed by the differences between studies in instrumentation, use of targeted vs untargeted methods, and measurement of polar vs nonpolar metabolites, as this results in a high variability in the types of metabolites screened, instrument sensitivity, and data acquisition and processing methods that can cause some potential biomarkers to go undetected. Differences in GDM diagnosis, with each study using a variety of diagnostic methods, such as those from the International Association of the Diabetes and Pregnancy Study Groups, American Diabetes Association, and World Health Organization, creates further difficulties in making direct comparisons between studies.

Additionally, information regarding GDM treatment was often not provided in postdiagnosis GDM studies, therefore limiting valuable information regarding the impact these treatments may have had upon metabolite levels. However, based on the sustained alterations observed in these potential biomarkers, a few common themes emerged. As discussed previously, elevated FFAs, pyruvate, citrate, and hypoxanthine, as well as decreased SMs, appear to respond to exercise and weight loss in nonpregnant populations [61, 62, 86, 87, 100, 101, 114]. An increase in physical activity is recommended upon GDM diagnosis, which takes place between 24 and 28 weeks, so the persistent alteration in these metabolites after diagnosis suggests that lifestyle changes are not occurring soon enough to rectify the metabolic perturbations associated with these dysregulated metabolites. A search for exercise metabolomics studies to verify the length of time needed to observe sustained improvement in the metabolome found that most studies investigate acute responses to short-term exercise rather than responses to sustained physical activity changes [115]. However, of the long-term exercise studies, an 80-day aerobic exercise intervention found a decrease in fatty acids and ketone bodies [116], while an 8-week anaerobic exercise intervention found reductions in BCAAs [117]. As such, early pregnancy or prepregnancy physical activity intervention may be necessary to normalize metabolite levels. Similarly, improvements in dietary intake may not be occurring early enough in pregnancy, suggesting early or prepregnancy dietary intervention as a method of metabolite normalization.

Another common theme involves interventions that induce metabolite alterations seemingly contradictory to what is proposed in this review. Metformin treatment compared to insulin treatment in those with GDM, for example, appears to increase BCAAs and VLDL that is commonly associated with elevated FFAs [66, 118]. Further studies investigating pharmaceuticals and potential GDM biomarkers may help uncover treatments that normalize levels of the metabolites focused upon in this review.

Similarly, a lack of GDM-specific dietary guidelines also results in dietary interventions that may be ineffective. The low-carbohydrate diet that is often prescribed for those with GDM may result in a diet high in fat, which may further contribute to elevated FFAs and increased insulin resistance. A diet low in BCAAs may aid in the reduction of BCAAs [67, 68] and therefore insulin resistance. However, low BCAA intake is not a current GDM dietary recommendation and whether this diet is safe for pregnancy is unclear, highlighting the need for further research. A Mediterranean diet appears to reduce risk of GDM [119], potentially through reducing PLA2 activity and normalizing oxylipin levels [81] or through lowering BCAA levels via reduced intake of animal proteins, as BCAAs are associated with higher intake of animal protein [120]. However, this diet is not a standardized intervention and research surrounding the Mediterranean diet, GDM, and potential GDM biomarkers is limited. Another diet that may be helpful in targeting these dysregulated metabolites is the low glycemic index diet, which is often prescribed to women with GDM and has shown some promise in GDM management [12]. In addition to reducing postprandial hyperglycemia, this diet may also reduce BCAAs [121]. Similar to dietary intervention, metabolomics research relating to supplementation as a GDM intervention method is also limited. Supplementation with fish oils, which might be expected to help normalize oxylipin levels, has not been shown to reduce risk of GDM [122, 123]. Other potential interventions, such as vitamin D supplementation, calcium supplementation, or probiotics, are not well researched and information regarding their effectiveness is limited [124]. Future GDM studies investigating the impact of different intervention methods on metabolite levels are necessary to understand the sustained alterations discussed in this review.

Another limitation of GDM metabolomics studies is lack of clarity regarding exclusion criteria. Of the 27 GDM metabolomics studies included in this review, 19 stated T1DM and

T2DM as an exclusion criterion [21, 22, 24–28, 32–34, 36, 39–41, 43, 44, 45, 88, 125], while 8 did not explicitly state these forms of diabetes as an exclusion criterion [14, 15, 17, 35, 38, 46–48]. Additionally, only 2 of these studies stated that pregestational diabetes was screened for during the study [44, 125], while all others appeared to use self-reporting or medical history to determine T1DM and T2DM status. This makes it possible that some GDM cases were previously undiagnosed T1DM or T2DM rather than true GDM. To gain more complete insight into GDM-specific metabolic perturbations, future GDM studies should be more rigorous in excluding patients with T1DM or T2DM.

Due to the limitations of the metabolomics studies discussed above, it is difficult to determine the cause and effect relationship between these metabolite alterations and GDM. Increases in ketone bodies, alpha-hydroxybutyrate, pyruvate, and citrate appear to occur as the body adapts to increasing insulin resistance, whereas elevated FFAs may originally be an adaptive change that then further contributes to inflammation and insulin resistance. Similarly, alterations in the purine degradation pathway and metabolites associated with the gut microbiome seem to increase inflammation and insulin resistance and therefore may contribute to GDM development or severity. The increase in BCAAs may be a driver behind GDM through the mTORC1 signaling pathway, and dysregulation of PCs, LPCS, and SMs may alter cell membranes and disrupt cell signaling in a process that contributes to GDM development.

In addition to a clearer delineation between GDM and other forms of diabetes, it is also important to characterize metabolite alterations that are due to GDM rather than due to pregnancy. While all studies discussed in this review compared metabolites altered across pregnancy in women with GDM compared with pregnant euglycemic women, suggesting that metabolic perturbations were due to dysglycemia and not pregnancy, we sought to further verify these changes through non-GDM metabolomics studies. One study of pregnant women with pregestational diabetes (TIDM or T2DM) compared with healthy pregnant controls found glutamine to decrease, while finding isoleucine and several ketone bodies to increase [24]. This is in line with our reported findings in GDM groups, suggesting that metabolite alterations are due to dysglycemia. Furthermore, a recent systematic review of plasma and serum metabolites altered in T2DM found BCAAs, alpha-hydroxybutyrate, lactate, and pyruvate to be elevated in T2DM, while glutamine decreased [126], all results that agree with our findings in GDM. However, future GDM studies comparing GDM pregnant women, non-GDM pregnant women, dysglycemic nonpregnant women, and euglycemic nonpregnant women would provide further valuable insight into metabolic perturbations influenced by dysglycemia.

Conclusions

Metabolites associated with GDM prediction were observed to remain altered across the course of pregnancy. The studies presented in this review reveal that GDM development is associated with metabolic perturbations related to BCAA metabolism, fatty acid oxidation, reductive carboxylation, cell membrane lipid metabolism, purine degradation, and the gut microbiome. Many of these pathways are associated with increased inflammation, insulin resistance, and share similarities with the progression of T2DM. Further, the study results discussed may identify metabolites potentially clinically valuable for earlier diagnosis and improved intervention. Further research is necessary to understand how postdiagnosis GDM interventions affect the metabolome. Additionally, early pregnancy or prepregnancy intervention studies are required to investigate whether normalization of these perturbed pathways would affect GDM development and outcomes. The importance of early detection and intervention is underscored by the numerous negative health outcomes associated with GDM, both during and after pregnancy, for the mother and child.

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Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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